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Title:
Hippocampal segmentation in older people with memory problems

HIPPOCAMPAL SEGMENTATION IN OLDER PEOPLE WITH MEMORY PROBLEMS

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Abstract

Early biomarkers of progressive and irreversible diseases like Alzheimer's disease (AD) are vital. The hippocampus is a structure heavily implicated in early AD pathology but is not a heterogenous structure. Segmentation of the hippocampus, into head, body and tail along the longitudinal axis, or into subfields, may help our understanding of prodromal AD. This study employed MRI to investigate brain changes in individuals with subjective cognitive decline (SCD) and mild cognitive impairment (MCI), compared to healthy controls. Subregion volume was measured, along with T2 relaxation time to investigate tissue integrity. No measurable differences were found between those with SCD and healthy controls, but groupwise differences were found in those with MCI compared to those without. These differences were found in CA1, dentate gyrus and subiculum volumes, as well as whole hippocampus, and CA1, CA3, dentate gyrus and subiculum T2 distributions, again as well as whole hippocampus. When classifying these groups, subiculum volume and T2 emerged as the best at distinguishing those with MCI from those without. Using data from a year follow-up session, it was found that volumes and T2 distribution related to cognitive score at baseline, but neither were able to predict decline that had occurred by the following year. Overall segmentation does not seem justifiable for use on diagnosis but using segmentation in combination with T2 relaxometry and potentially functional connectivity could tell us about small changes in tissue pathology in the early stages of AD. The importance of the hippocampal head should be investigated over a longer time period.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is my own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the UK, affecting over 500,000 people in the UK (Prince et al., 2014). In addition, the human impact of diseases, dementia currently costs the UK £26 billion, and this is projected to more than double in the next 25 years (Lewis, Karlsberg Schaffer, Sussex, O'Neill & Cockcroft, 2014; Prince et al., 2014). Despite increasing amounts of research focusing on dementia, it is the only condition in the top ten causes of death in the UK without a treatment to prevent, cure or slow its progression (Office of National Statistics, 2017; National Records of Scotland, 2017; Northern Ireland Statistics & Research Agency, 2016).

AD is a progressive neurodegenerative disease. Although the exact pathology of the disease is still somewhat unknown, the proteins tau and β-amyloid (Aβ) are implicated as the neurofibrillary tangles and plaques they produce have been found in high levels at post-mortem in people with AD (Braak & Braak, 1991; The National Institute on Ageing et al., 1997). Apolipoprotein E (APOE), particularly the ε4 allele has been found to be a major risk factor in both late- and early-onset AD and is thought to underlie the pathophysiology of AD (Chartier-Hariln et al., 1994).

Despite this knowledge, drug discovery research has been largely unsuccessful; between 2002 and 2012, the success rate for Alzheimer's disease trials was 0.4% (Cummings, Morstorf & Zhong, 2014). The success rate for advancing through phases is low and the number of compounds progressing to regulatory review are one of the lowest found in any therapeutic area (Cummings et al., 2014). It is possible that the ability of drugs to stop progression is only effective in the early stages of the disease. Jack and colleagues (2010) suggest that biochemical and subsequently structural changes occur in the brains of those with AD long before clinical manifestation of symptoms. These early pathological hallmarks can occur decades before a formal diagnosis is made. It is likely that developing an early biomarker to identify the early pathology of incipient AD will not only aid understanding of the mechanisms of the disease, but also identify a new patient cohort for clinical trials aiming to develop potential treatments.

Mild cognitive impairment (MCI) has been widely studied as an incipient form of dementia, with a 10–15% annual risk of converting to AD (Petersen et al., 1999). MCI is decline detectable in standardised tests of memory, but not warranting a clinical diagnosis of AD. Individuals with MCI typically present with impaired episodic long-term memory, but not short-term or implicit memory (Perri, Carlesimo, Serra & Caltagirone, 2005). MCI patients

who convert to AD over a period of two years, tend to perform worse at initial testing than those who do not (Perri, Serra, Carlesimo & Caltagirone, 2007). This trend is found across episodic memory indexes, e.g. learning, forgetting and recognition. Patients with MCI show early pathology of AD, for example an abnormal ratio of 42 amino acid Aβ to tau in their cerebrospinal fluid (CSF), similar to an AD profile (Visser et al., 2009).

It has been suggested that AD is a three-stage process with AD preceded by MCI and MCI preceded by subjective cognitive decline (SCD; Jessen et al., 2010). People with SCD report memory problems, however these deficits are not detectable through standard cognitive tests (Jessen et al., 2014). Despite this lack of objective deficit, older people with SCD show 4.5 times the risk of gaining an MCI or dementia diagnosis, and decline more rapidly, than those without SCD (Reisberg, Shulman, Torossian, Leng & Zhu, 2010). There have also been biological changes detected in those with SCD such as early Aβ pathology (Perrotin, Mormino, Madison, Hayenga & Jagust, 2012). A meta-analysis estimated that over a 4-year period, 25% of those with SCD convert to MCI (Mitchell, Beaumont, Ferguson, Yadegarfar & Stubbs, 2014). It has since been suggested that conversion for the SCD group as a whole is more nuanced, with memory decline in the first two years being predictive of conversion to AD, with other remaining stable (Hessen et al., 2017).

SCD is likely to be a complex phenomenon with factors such as education level (van Oijen, de Jong, Hofman, Koudstaal & Breteler, 2007) and concurrent medical conditions such as depression (O'Connor, Pollitt, Roth, Brook & Reiss, 1990) increasing prevalence of SCD. It is likely that studying pathology in those with MCI and SCD will provide insights into early AD changes and may yield a biomarker for the disease.

1.1 Brain structure

Structural MRI can provide accurate measures of brain atrophy, typically caused by a loss of neurons and synapses. Post-mortem neuronal counts establish MRI as an accurate measure of brain volumes (Bobinski et al., 1999). Volumetric MRI measures reveal a strong relationship between brain atrophy and cognitive impairment in healthy controls (HCs), people with SCD, MCI and AD (Jack, Petersen, O'Brien, & Tangalos, 1992). Whilst global gray matter atrophy is to some extent normal in healthy ageing (see Peters, 2006), an accelerated pattern of atrophy is predictive of AD (Jack et al., 2012). MRI can detect early changes in tissue volume and integrity and is viable for diagnostic use (Knight, McCann, Kauppinen & Coulthard, 2016). Whilst whole brain volume can be useful at distinguishing those with a diagnosis of AD from healthy older adults, investigating regions affected by early pathology can help identify those with incipient AD. Aβ changes in AD are seen across

the cortex, however tau pathology starts in the medial temporal lobe (MTL) and progresses to the cortical association areas and finally the whole brain (Thal, Rüb, Orantes & Braak, 2002; Delacourte et al., 1999).

Since the seminal study of HM (Scoville & Milner, 1957), the MTL (consisting of the hippocampus, perirhinal, entorhinal and parahippocampal cortices; Squire, Stark & Clark, 2004) has been heavily implicated in the process of memory consolidation (Walsh et al., 2014). This brain region has been long established as a region of interest in AD, with significant progressive atrophy a defining characteristic of the disease (Jobst et al., 1994). The MTL is also the earliest affected brain region in patients with MCI (Pennanen et al., 2005; Pihlajamaki, Jauhiainen & Soininen, 2009) and rate of atrophy in the MTL predicts conversion from MCI to AD (Visser, Verhey, Hofman, Scheltens & Jolles, 2002; Korf, Wahlund, Visser & Scheltens, 2004). Research into older adults with SCD shows mixed results when investigating brain structure. Saykin and colleagues (2006) found that whilst there may be small changes in MTL gray matter volumes in those with SCD, only those with MCI show significant atrophy when compared to healthy controls. The degree of atrophy in the MTL correlated with the extent of memory complaints and performance deficits. It is likely that early changes are too small to be identified across the MTL and that specific regions are more vulnerable to AD pathology.

The hippocampus, in particular, is thought to be vital in supporting declarative recollection memory processes (Wolk, Dunfee, Dickerson, Aizenstein & DeKosky, 2011). Removal of or lesion to the hippocampus results in loss of episodic memory, spatial learning and contextual fear, suggesting that it is a key brain region in the domains of learning and memory (Fanselow & Dong, 2010). Hippocampal volume is strongly correlated with memory performance (Köhler et al., 1998) and can distinguish individuals with AD from healthy controls (Jack et al., 1992). Those with MCI also show decreased volumes in MTL structures including the hippocampus (Schuff et al., 2009). Furthermore, older adults who go on to convert to AD show accelerated atrophy in this region (Sabuncu et al., 2011).

1.2 Segmenting the hippocampus

In current clinical practice, volumetric imaging of the whole brain and whole hippocampus is used to diagnose and monitor AD progression. Studies report hippocampal volume reduction of 24% in those with AD and 12% in those with MCI (Shi, Liu, Zhou, Yu & Jiang, 2009). Despite significant hippocampal volume changes at these stages of disease, these volumes are not sensitive enough to predict which MCI patients will convert to AD or consistently

present in SCD (Saykin et al., 2006). It is possible that early brain pathology is localised to specific subregions of the hippocampus. Different subregions of the hippocampus have different cellular composition, gene expression and projections to different areas (see Fanselow & Dong, 2010).

Traditionally, hippocampus has been distinguished along the longitudinal axis into anterior hippocampus (AH) and posterior hippocampus (PH) regions, divided by the uncal apex (Poppenk, Evensmoen, Moscovitch & Nadel, 2013). Lesion studies in rodents focus on the anatomically analogous ventral (VH) and dorsal (DH) regions of the hippocampus respectively (Strange, Witter, Lein & Moser, 2014). These studies describe functional distinctions, where lesions of the VH, but not the DH impair stress response and emotion production (Henke, 1990). Meanwhile, in humans, AH lesions selectively impair memory, particularly spatial memory (Maguire et al., 2000). The PH communicates to the visual, auditory and somatosensory cortices, whilst the AH projects indirect and direct connections with amygdala, hypothalamus and subcortical nuclei involved in autonomic regulation (Moser & Moser, 1998; Witter, 2009; Fanselow & Dong, 2010). The AH, situated next to the amygdala, is functionally associated with emotion, specifically anxiety-related behaviours (Kjelstrup et al., 2002; Bannerman et al., 2004).

Segmenting the hippocampus along the longitudinal axis in this way has produced some inconsistent findings. In healthy populations, some studies show AH atrophy as individuals grow older (Jack et al., 1997; Chen, Chuah, Sim & Chee, 2010; Rajah, Kromas, Han & Pruessner, 2010), however some studies implicate PH volume loss (Kalpouzos et al., 2009; Raji, Lopez, Kuller, Carmichael & Becker, 2009) and there is also evidence that both regions are affected in healthy ageing (Pruessner, Collins, Pruessner & Evans, 2001). In AD, hippocampal atrophy is usually more pronounced in the AH (Jack et al., 1997; Malykhin, Bouchard, Camicioli & Coupland, 2008), with AH volume even indicating which individuals will go on to develop MCI (Martin, Smith, Collins, Schmitt & Gold, 2010). This could act as a biomarker of early AD; however, it is important to note that there is overlap between healthy ageing and AD pathology.

An alternative to anterior/posterior segmentation is segmenting the hippocampus into head, body and tail (HBT) regions (see Figure 1a for illustration). Head, body and tail show differential patterns of atrophy in early AD and also have differing relationships with other biomarkers, i.e. neuropsychology and CSF-base tau and Aβ markers (Greene & Killiany, 2012). These subregions of the hippocampus also have distinct projections: the head to the prefrontal cortex; the body to the posterior cingulate cortex; the tail to the amygdala (Zarei et

al., 2013). There is evidence for a functional distinction between the hippocampal head, body and tail. In general, head volume correlates with verbal memory performance, whilst body and tail volumes correlate with visual memory (Travis et al., 2014).

Gordon, Blazey, Benzinger and Head (2013) explored the inconsistency of AH-PH findings by using a large sample of older people and HBT segmentation. They found that the hippocampal head and body showed more atrophy in healthy ageing than the tail. They also reported that the hippocampal head is most affected in AD. More interestingly, they found that volume loss localised to the head of the hippocampus, occurs with increasing severity of dementia. This is not a phenomenon seen in the hippocampal body or tail. Atrophy of the head and body is also increased in those with MCI (Martin, Smith, Collins, Schmitt & Gold, 2010). Greene and Killiany (2012) found that the hippocampal head was the only region correlated with levels of tau in the brain, but Aβ level was associated across the longitudinal axis. They concluded that head volume, in conjunction with presence APOE alleles and neuropsychology could predict those with MCI who went on to develop AD, but this was not possible by only using HBT volumes.

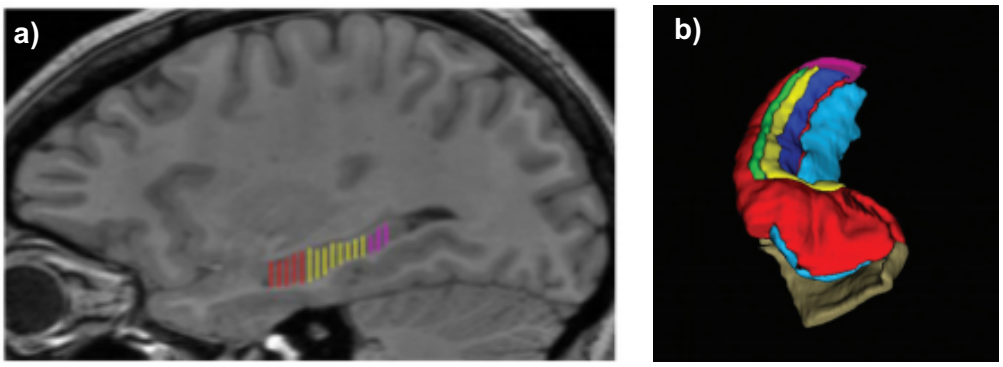

Figure 1. Figure a) shows ● head, ● body and ● tail regions of the hippocampus within the human brain (taken from Daugherty et al., 2015). Figure b) shows a manual mask taken from Wisse and colleagues (2016): ● CA1; ● CA2; ● CA3; ● dentate gyrus; ● subiculum; ● entorhinal cortex; ● tail.

The hippocampus can also be split into subfields: the cornu ammonis fields (CA1-3), dentate gyrus (DG) and the subicular complex (including subiculum proper, presubiculum and parasubiculum; see Figure 1b). Anatomically, all subfields are to some extent situated within the hippocampal head, body and tail, however a majority of CA1-3 are situated in the head and dentate gyrus is mostly in the body (Malykhin, Lebel, Coupland, Wilman & Carter, 2010). Functionally, anterior DG and CA volumes have been implicated in verbal memory, whilst posterior DG/CA volumes are linked to spatial memory (Travis et al., 2014). The subfield CA1 plays a key role in communication between the cortex and the hippocampus, necessary for consolidation and therefore the formation of long-term memories (Remondes & Schuman, 2004). The subiculum is another subregion that may be of interest in early AD.

Smaller subiculum volume is associated with poorer cognition, specifically executive dysfunction. Shrinkage of this area is also linked with increased risk of dementia (Evans et al., 2018).

Hippocampal volume loss, especially in the subfields CA1 and subiculum, is an early feature of AD (De Flores, La Joie & Chételat, 2015). It is suggested that AD pathology first affects the entorhinal cortex, with significant atrophy occurring in the subiculum and CA1 next (Apostolova et al., 2010; Mueller et al., 2010). This atrophy in subiculum and CA1 increases with severity of condition, with small changes sometimes seen in those with SCD, through to larger changes in MCI and AD (Zhao et al., 2019). Csernansky et al. (2000) concluded that damage to the head and lateral surface of the hippocampal body, corresponding to anterior CA1, is characteristic of AD. Most research has focused on CA1 as the key subfield affected by AD pathology. There is marked atrophy in CA1, with 22% loss in volume in those with amnestic MCI and 27% in those with AD (La Joie et al., 2013). Some atrophy of the hippocampus is normal in healthy ageing (see Zheng et al., 2018), however investigations into specific subregions has provided more sensitive biomarkers in those with prodromal AD (Mueller et al., 2010; Pluta, Yushkevich, Das & Wolk, 2012; La Joie et al., 2013). These researchers found that CA1 volume best discriminates individuals with MCI from HCs, better than whole hippocampal volume. Despite the increased sensitivity of CA1, whole hippocampal volume still shows a moderate ability to distinguish groups (Pluta et al., 2012).

1.3 T2 relaxometry

In addition to volumetry, T2 relaxation time (T2) is another measure which could reveal early pathology of AD. T2 (also referred to as ‘spin-spin’ or ‘transverse relaxation time’), can be used to identify microstructural tissue changes caused by disease (see Su et al., 2016). This measure is produced by using a radiofrequency pulse to disrupt the spin of protons in tissue, causing them to spin in phase. By recording the time taken for the spins to return to equilibrium, we can make inferences about the properties of the tissue (Knight, McCann, Kauppinen & Coulthard, 2016). Water content, myelin density and the presence of paramagnetic molecules all influence T2. Tissue with a short relaxation time can be caused by densities such as iron deposits, and particularly in AD, Aβ plaques and neurofibrillary tangles; whereas high T2 could indicate oedema and excess CSF (Tang et al., 2018). Volume changes are a result of atrophy that has occurred as a result of AD, whereas T2, when used as a proxy for tissue integrity and health, could highlight incipient AD before major changes to the brain occur (Knight et al., 2016).

T2 relaxometry has been employed in disease research, most notably in the investigation of temporal lobe epilepsy (see Bernasconi et al., 2000; Von Oertzen et al., 2002). T2 has been investigated less in early AD, as the hippocampus can sometimes be prone to worse signal to noise ratio, as due to its position in the middle of the human head. Despite this, a growing body of literature suggests this may be a useful biomarker. Granziera and colleagues (2015) described a pattern of increased T2 in the hippocampi of MCI patients compared to HCs. The researchers attributed this increase to demyelination and loss of cellular proteins and tissue microstructure. A decrease in T2 was found in the globus pallidus, suggesting iron accumulation localised to this region. There is also evidence that these biological changes reflect functional abilities. Wood, Saling, O'Shea, Berkovic and Jackson (2000) reported that high T2 in the hippocampus correlates with decreased memory and executive function abilities.

In a review of relaxation time in those with AD, Tang and colleagues (2018) highlight the conflicting findings in the literature. Many human studies find an increase in hippocampal T2 in those AD and probable AD (e.g. Kirsch, Jacobs, Butcher & Beatty, 1992; Wang, Yuan, Shu, Xie & Zhang, 2004; Raven, Lu, Tishler, Heydari & Bartzokis, 2013). Despite these reproduced findings, some studies seemingly conclude the opposite: that T2 is decreased in the hippocampi of those with AD (e.g. Luo et al., 2013; Su et al., 2016). This is further clouded by findings in mouse models of AD, which almost always show decreases in T2 (see Helpern et al., 2004; Falangola et al., 2007; Teipel et al., 2011; Kara et al., 2015).

A potential explanation for these conflicting findings is that research in this area uses mean T2 as a measure. As introduced earlier, both low and high T2 are abnormal and signs of disease. To recap, “tissue with a short relaxation time can be caused by densities such as iron deposits, and particularly in AD, Aβ plaques and neurofibrillary tangles; whereas high T2 could indicate oedema and excess CSF (Tang et al., 2018)”. Therefore, both low T2, due to protein accumulation, and high T2, due to atrophy, are present in AD. It is possible that decreases in T2 are specific to AD, whereas T2 increases in AD but also in healthy ageing. Following this logic, an AD profile will more typically show a wide T2 distribution, reflecting unhealthy tissue on each end of the distribution. It is important to focus on T2 distribution, as this provides information not accurately reflected in the mean value of T2. Changes in this novel marker of brain integrity, T2 relaxation time, may be used in conjunction with different hippocampal segmentation methods to help better understand AD pathology. Variability of T2 within subfields show strong differences between HCs and individuals with MCI (Knight, Wearn, Coulthard & Kauppinen, 2018). It is likely that healthy individuals typically show a

more narrow T2 distribution, clustered around the mean, whereas those with AD pathology have a wider distribution, caused by unhealthy tissue on both tails of the distribution.

1.4 Research aims

This research project aims to investigate the utility of segmenting the hippocampus by subfield and along the longitudinal axis through head, body and tail segmentation in the context of prodromal AD. T2 distribution of hippocampal subfields will be employed, alongside volumetric measures. This will provide support for segmentation of the hippocampus as a useful tool for understanding AD, above what is currently gleaned from whole hippocampal investigations. The research will investigate which segmentation methods, if any, are useful at distinguishing those with SCD and MCI from HCs.

1.4.1 Hypotheses

1. The hippocampal head will be most powerful in classifying patient groups, with this effect diminishing towards the tail.
2. CA1 and subiculum will be particularly useful in classifying patient groups and suggest early pathology in these areas.
3. These classifying subregions will predict cognitive decline after a period of one year.

Author's contribution

The author of this dissertation conducted all the included analyses, in addition to collecting the follow-up data for the project.

2. Methods

2.1 Participants

2.1.1 Recruitment

Participants were recruited from community settings: the ‘Join Dementia Research’ website; Avon and Wiltshire mental health partnership ‘Everyone Included’ recruitment system; local GP surgeries and memory clinics; the ‘ReMemBr Group’ volunteer database and word of mouth.

Inclusion criteria:

- A minimum age of 60 years was used as a guide for inclusion in the study. Efforts were made to age-match healthy controls (HCs) and participants with SCD to those with MCI. This caused some exceptions to the 60-year cut-off, due to a participant with MCI recruited who was 53.
- Participants who were concerned about their memory (except for HCs).
- No diagnosis of dementia.
- No known neurological disorders.

2.1.2 Participant group classification

Participants in the MCI and SCD participants were assessed over the telephone for eligibility, to ascertain whether they had a subjective memory complaint. Before testing for objective impairments, it was important to ascertain the participants’ subjective opinion of their own memory decline. This forms the subjective component of SCD.

This presence of SCD was determined by three questions based on the accepted SCD framework outlined in Jessen et al. (2014):

1. Are you concerned about your memory?
2. Do you think your memory is worse than it was 5 years ago?
3. Do you think that your memory is poorer than other people of a similar age?

Participants were required to answer yes to two of the questions to be classified as having the presence of SCD.

Figure 2 shows the classification pipeline used. Participants were assigned to either the SCD or MCI group based on their cognitive abilities (Addenbrooke’s Cognitive Examination;

Hsieh, Schubert, Hoon, Mioshi & Hodges, 2013), memory performance (Rivermead Behavioural Memory Test; Wilson, Cockburn & Baddeley, 2003) and appraisal of severity of dementia-related symptoms (Clinical Dementia Rating; Morris, 1997). Detailed descriptions of these neuropsychological tests are presented in section 2.2.2.

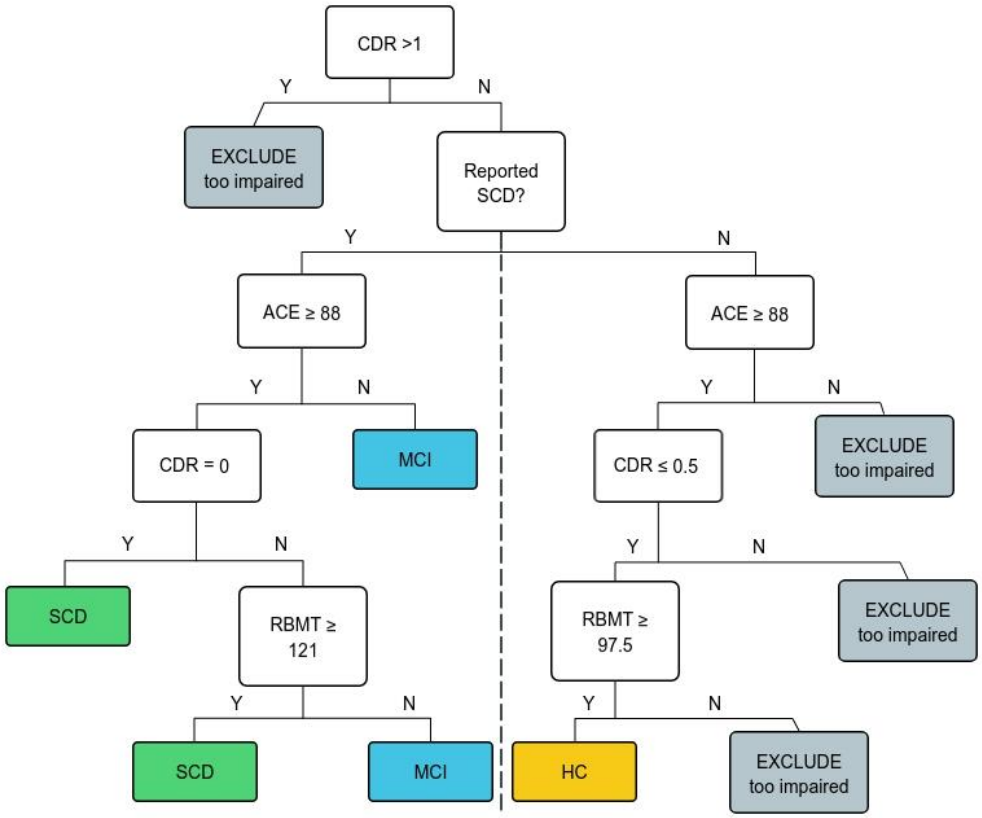

Figure 2. Flow chart showing the criteria by which participants were classified as HCs, individuals with SCD, or MCI within the current research study. Definitions were necessary as classification of MCI and SCD can differ between clinics/labs. HC = Healthy Control; SCD = Subjective Cognitive Decline; MCI = Mild Cognitive Impairment; ACE = Addenbrookes Cognitive Examination-III; RBMT = Rivermead Behavioural Memory Test 3; CDR = Clinical Dementia Rating.

Initial screening used CDR scores; any participant with a score of >1 was excluded as this was indicative of dementia, rather than an early stage problem. Importantly, the presence of SCD reported by the participants was used to identify HCs, they must then show no objective signs of impairment to be included in the study. The ACE threshold of 88 was defined from research into the upper limit for objective signs of cognitive impairment (Hsieh et al., 2013). RBMT scores were scaled as outlined in section 2.2.2 of this report. The upper and lower RBMT thresholds were chosen as they represent 1 (121) and 2 (97.5) standard deviations below the standardised mean.

Participants who reported memory problems that were also found objectively through the ACE, CDR and RBMT were classified in the MCI group. Those who reported memory

problems but did not show objective impairment measured by the ACE, CDR and RBMT, were classified in the SCD group. The groups were defined purely by their psychological test scores; this was invariant of any clinical diagnosis they may have received. Those who had no subjective or objective memory problems acted as HCs.

Clinical diagnosis is typically based on concern regarding a change in cognition and impairment in one or more cognitive domains (e.g. tested with ACE or MMSE) but preservation of independence in functional abilities and no evidence of dementia (e.g. tested with CDR; Albert et al., 2011). In this study, medical records were not accessed, and data was not shared with the participant's GP unless there was a specific concern found through the MRI scan.

2.1.3 Participant demographics

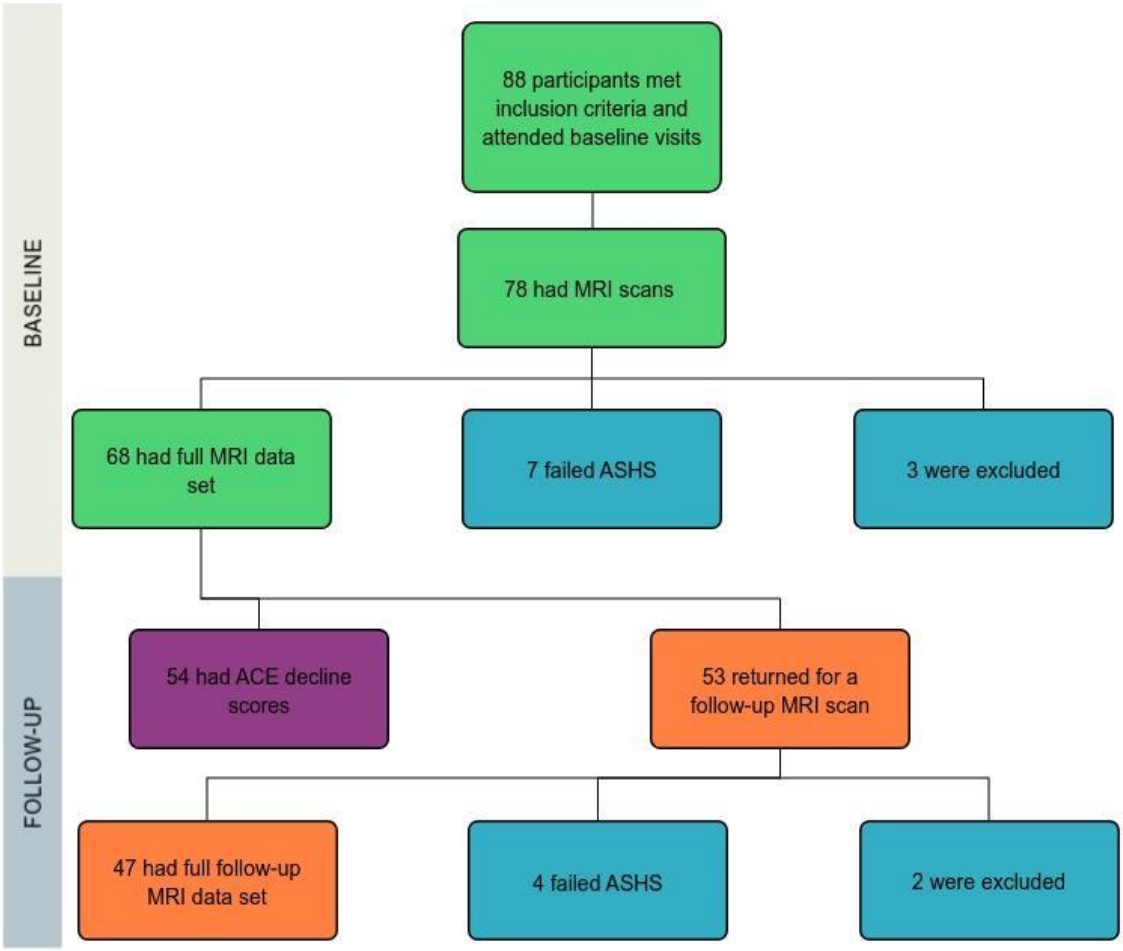

Figure 3. Participant flow throughout HIPPO2. ACE = Addenbrooke's Cognitive Examination III; ASHS = Automatic Segmentation of Hippocampal Subfields.

As can be seen in Figure 2, 10 participants were enrolled in the study but did not have imaging data due to MR contraindications. At baseline, 3 participants were excluded due to

the participant classification process described in Figure 1. Image quality was checked for signal to noise. The Automatic Segmentation of Hippocampal Subfields (ASHS; Yushkevich et al., 2010) program used to process the MRI scans failed to mask correctly for 7 participants at baseline. This was identified through visual inspection of the masks and was present on scans with the most atrophy. This was due to the training set (consisting of HC and MCI atlases) not being representative of the scans for those individuals who had the most damage.

As a result, 68 participants across the patient groups underwent an MRI scan with successful subsequent segmentation processes and were used for baseline analyses. The sample consisted of 19 participants with MCI, 25 with SCD and 24 HCs. The mean age of the sample across groups was 71.8 ± 8.4 with a range of 53-89 years. The sample was 43% female. Participant age and gender broken down by patient group and can be found in Table 1. The MCI group was disproportionately comprised of more males than the other two groups.

Table 1. Baseline participant demographics by group.

	HC	SC	MCI
Baseline MRI (N=68)			
N	24	25	19
Age (years, M ± SD)	71.12 ± 7.93	70.48 ± 7.82	74.53 ± 9.50
Female % (N)	45.8% (11)	48.0% (12)	31.6% (6)

Analyses looking at change over time employed further data from testing sessions after a period of 1 year. 54 of the above participants returned to repeat cognitive testing follow-up and 53 returned for a follow-up MRI scan. Overall there was a retention rate of 78-79% for MRI and cognitive testing respectively. Those with SCD in particular were likely to have a vested interest in returning, as they were concerned about their memory. Retention rate was much lower in the MCI group, with a retention rate of 42-53%. The ability of those with MCI to return was in some cases complicated by their impairment. Those in the MCI were also on average older, and some participants in this group were unwell with other conditions or passed away during the follow up period. At follow-up 6 participants did not have viable MRI data, resulting in 47 datasets for brain imaging-based analyses in this study.

The mean age of the follow-up MRI sample across groups a year later was 71.1 ± 8.0 with a range of 54-88 years. The follow-up MRI sample was 40% female. For follow-up cognitive testing, the sample had a mean age of 71.9 ± 8.3 with a range of 54-91 years. This cognitive follow-up sample was 41% female. Participant metrics by group for follow-up data can be seen below in Table 2.

Table 2. Follow-up participant demographics by group. Follow-up cognitive participants had Addenbrooke's-III (ACE) scores at baseline and follow-up, allowing an ACE decline score to be calculated. Follow-up MRI participants were those who returned for an MRI scan and the images passed successfully through the ASHS pipeline. Participants in the follow-up analyses were subsequently pooled into 'Non-MCI' and 'MCI' groups as no significant difference was found between HCs and SCDs at baseline.

	HC	SCD	MCI
Follow-up cognitive (N=54)			
N	21	23	10
Age (years, M ± SD)	71.19 ± 7.82	71.09 ± 7.80	75.50 ± 10.43
Female % (N)	42.9% (9)	43.5% (10)	30% (3)
Follow-up MRI (N= 47)			
N	18	21	8
Age (years, M ± SD)	70.28 ± 6.41	70.52 ± 7.84	74.63 ± 11.33
Female % (N)	44.4% (8)	38.1% (8)	37.5% (3)

2.2 Materials and study design

2.2.1 Design

Baseline

The baseline study consisted of 2 sessions, 4 weeks apart (± 2 days). The first session involved several cognitive tests. The participant was asked to bring a study partner in for this session. The study partner took part in the interview and questionnaire indicated by Δ in section 2.2.2. The MRI scan typically took place in session 2. Each session lasted approximately 2 hours.

Follow up

The follow up similarly consisted of 2 sessions on separate days, usually within a month of each other, but there were no fixed restrictions on the time between sessions. Again, the MRI scan typically took place in session 2. The testing sessions at follow up lasted approximately 1.5 hours.

Both baseline and follow up session protocols can be seen in Appendices A and B respectively.

2.2.2 Neuropsychological testing

Rivermead Behavioural Memory Test-3 (RBMT; Wilson et al., 2003): A test of memory performance through a variety of tasks. Participants were randomised to version 1 or 2 of the RBMT at baseline. In the follow up sessions, participants were tested using the alternative version to avoid any learning effects (Wills, Clare, Wills, Shiel & Wilson, 2000). The subtests of the RBMT can be seen below. The total maximum raw score was 212. Scores were scaled according to the tables in the RBMT administration and scoring manual (Wilson et al., 2003). This normalised the data to age, based on expected performance by HCs, and balanced the weighting of each test. Higher scores indicate better performance on the subtests. Tests 2, 3 and 8 were split into their prospective and retrospective memory components.

1. First and Second Names: At the beginning of the RBMT, participants are asked to remember the names of two people shown to them in photographic portraits. At the end of the test, the participant is shown the two photographs again and asked to recall their names. Full points can only be given if the participant is not prompted. Max score = 8.
2. Belongings: Next, participants were asked to give the experimenter two small items for the duration of the experiment. The two items were placed in two different locations in the testing room, out of sight of the participant. The participant is told to ask for the items back and tell the experimenter where the items are at the end of the test, when the experimenter says “we have finished this test”. Partial points were given if the participants needed prompting to give the information. Max score = 8.
3. Appointments: Early in the testing session an alarm is set for 25 minutes time. The experimenter asks the participant to ask them two predefined questions when the alarm sounds. Points are given for recall of the questions and remembering the correct task when the alarm sounds. Max score = 4.

4. Picture Recognition: The participants were shown 15 drawings and asked to name the object pictured. After a short delay, participants were presented with 30 drawings, half of which were from the initial set and half of which have not been seen before. Participants were asked to say whether they had seen each picture before or not. Max score = 15.
5. Story: The experimenter reads the participant a short newspaper item. The participant is then asked to recall as much of the story as possible.
    a. Immediate, max score = 21.
    b. Delayed, max score = 21.
6. Face Recognition: The participants were shown 15 photographs of faces and asked to say whether the person pictured is male or female and over or under 40 years of age. After a short delay, participants were presented with 30 photographs, half of which were from the initial set and half of which have not been seen before. Participants were asked to say whether they had seen each photograph before or not. Max score = 15.
7. Route: The experimenter performs a short route around the testing room, consisting of six locations. The participant is then asked to retrace the route. Points are given for each correct location visited and the order of the route.
    a. Immediate, max score = 13.
    b. Delayed, max score = 13.
8. Messages: During the route demonstration, the experimenter took two items with them. Full points can only be given if the participant is not prompted.
    a. Immediate, max score = 6.
    b. Delayed, max score = 6.
9. Date and Orientation: 13 questions testing the participant's orientation to time (e.g. what is the day of the week?) and place (e.g. what is the name of the place we are in?). The measure also included knowledge linked to time and place (e.g. what is the name of the current Prime Minister?). Max score = 14.
10. Novel Task: The experimenter demonstrated how six coloured pieces form a shape inside a template, using a set order. The participant then attempted to repeat the process. This was conducted three times to give the participant the chance to learn. Points were given for the correct positioning of each piece and the correct order.
    a. Immediate: three learning trials. Max score = 17 for each trial (51 total).
    b. Delayed, max score = 17.

Addenbrooke's Cognitive Examination-III (ACE; Hsieh et al., 2013): A test of general cognitive score commonly used in clinical settings (Newman et al., 2018). ACE tests the following cognitive domains, with examples given below.

1. Attention: (e.g. participants are asked to subtract 7 from 100 and keep taking 7 away from each new number until asked to stop). Max score = 18.
2. Memory (e.g. remembering a name and address after a short delay). Max score = 26.
3. Verbal fluency: (e.g. participants are given one minute to name as many words as possible beginning with the letter P). Max score = 14.
4. Language: (e.g. participants are asked to repeat the following words: caterpillar, eccentricity, unintelligible, statistician). Max score = 26.
5. Visuospatial abilities: (e.g. participants are asked to draw a clock face, with numbers, and the hands set at 10 past 5). Max score = 16.

The total maximum score is 100, with higher scores indicating better cognitive functioning. Participants were randomly assigned to version A or B of the ACE-III at baseline and the alternative version was used at follow up.

Δ Clinical Dementia Rating (CDR; Morris, 1997): A clinically used dementia severity rating tool, focusing on the domains of memory, orientation, judgement & problem solving, community affairs, home & hobbies and personal care. In this study, the CDR was used only for group classification.

Other tests carried out but not included in this dissertation

Paired Associates Learning (PAL; CANTAB®): Boxes are displayed on a tablet screen and disappear in sequence to reveal either a coloured pattern, or nothing, behind. The pattern(s) then appear in the middle of the screen and the participant must touch the box where they think they previously saw that pattern. If the participant makes an error, the sequence repeats. The test has 6 levels and a practice level, each with increasing difficulty.

Brief Cognitive Rating Scale (BCRS; Reisberg & Ferris, 1988): Part I of this test was used. This simple test is used to assess functional and cognitive abilities. This probes the domains of concentration, recent memory, remote memory, orientation, functioning & self-care.

Δ Activities of Daily Living Questionnaire (ADLQ-T; Muñoz-Neira et al, 2012): Study partners were given this questionnaire which measures the participant's functioning in the domains of self-care, household care, employment & recreation, shopping & money, travel, communication and technology.

Nottingham Extended Activities of Daily Living (NEADL; validated in stroke patients in Wu, Chuang, Lin & Hong, 2011): A questionnaire probing the everyday activities of the participant. Answers are categorised into 'not at all', 'with help', 'on my own with difficulty' or 'on my own'. Participants were asked to report their activity in the past few weeks, rather than their capabilities.

Depression Anxiety Stress Scales (DASS-21; Lovibond & Lovibond, 1995): A clinical measure of state depression, anxiety and stress.

California Verbal Learning Task- II (CVLT-II; Delis, Kaplan, Kramer & Ober, 2008): At baseline testing, a word list was constructed based on the CVLT. Participants were tested on recall and recognition of the words at 30-minutes, 24-hours and 4-weeks after initial learning.

Rey Auditory Verbal Learning Task (Schmidt, 1996): At follow-up, a word list was adapted from this. The word list consisted of 15 words, which were repeated until a threshold of 75% correct was demonstrated (a minimum of 4 trials or maximum of 10 trials). Participants were tested on recall and recognition of the words at 30-minutes, 24-hours and 7 days.

Rey-Osterrieth Complex Figure Test (Fastenau, Denburg & Hufford, 1999): Participants are asked to copy a complex figure and later reproduce it from recall. This test was used only at baseline.

2.2.3 Magnetic resonance imaging

Image acquisition

This protocol is taken from Wearn (submitted), which used the same protocol.

Imaging data at both time points was collected using the same Siemens Magnetom Skyra 3T system at the Clinical Research Imaging Centre (CRIC) in Bristol. 32-channel head receiver array coil and parallel transmit body coil. Scan images were inspected by the MRI operator during the scan to check for movement artefacts. If time allowed, a suboptimal scan was repeated.

3D T1-weighted MPRAGE: Sagittal, TR 2200 ms, TE 2.28 ms, TI 900 ms, flip angle 9°, FOV 220 x 220 x 179 mm, acquired resolution 0.86 x 0.86 x 0.86 mm, acquired matrix size 256 x 256 x 208. Acquisition time: 5:07 min.

High-resolution hippocampal turbo spin-echo. These sequences were localised to approximately 1 cm anterior and posterior to each pole of the hippocampus, therefore they did not provide whole brain coverage.

> Multi-contrast TSE: Coronal, TR 7500ms, number of echoes: 3, TE 9.1, 72 & 136 ms, acquired resolution 0.69 x 0.69 x 1.5 mm, reconstructed resolution 0.34 x 0.34 x 1.5 mm (after 2-fold interpolation in-plane by zero-filling in k-space, and inclusive of 15% slice gap), GRAPPA factor 2, FOV 220 x 220 x 34, acquired matrix size 270 x 320 x 58. Acquisition time: 5:09 min.
>
> Single-contrast TSE: Coronal, TR 3200 ms, TE 60 ms, acquired resolution 0.69 x 0.69 x 1.5 mm, reconstructed resolution 0.34 x 0.34 x 1.5 mm (after 2-fold interpolation in-plane by zero-filling in k-space, and inclusive of 15% slice gap), GRAPPA factor 2, FOV 220 x 220 x 34 acquired matrix size 270 x 320 x 58. Acquisition time: 3:17 min.

Scans conducted but not used in this dissertation

Whole brain CPMG, acquisition time: 7:09 min.

DTI, acquisition time: 3:15 min.

2.2.4 Segmentation methods

ASHS

Hippocampal subfield segmentation was performed using ASHS (Automatic Segmentation of Hippocampal Subfields; Yushkevich et al., 2010). ASHS uses a T1-weighted (MPRAGE) and T2-weighted (single-echo and 3TE) scans to automatically mask the hippocampus and subregions. ASHS also reports intracranial volume (ICV). ASHS has been found to be robust (Xie et al., 2018). Figure 3 shows the masking output of ASHS, segmenting the subfields CA1-3, dentate gyrus, subiculum, entorhinal cortex and perirhinal cortex.

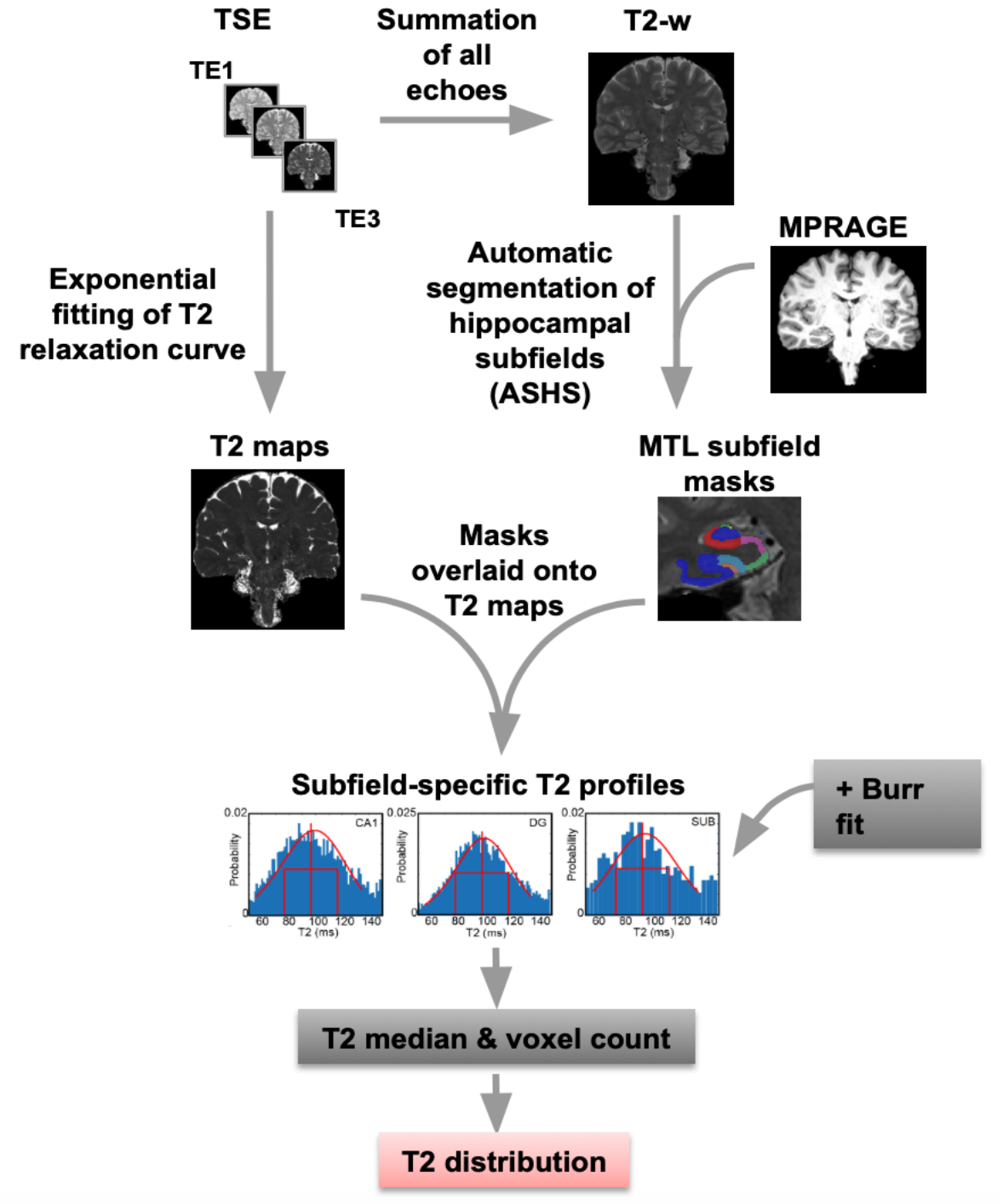

Figure 4. A visual representation of the ASHS pipeline, used to segment the hippocampus into subfields and produce T2 distribution for use in this study. The ASHS programme also used the MTL subfield masks to report volumes and ICV.

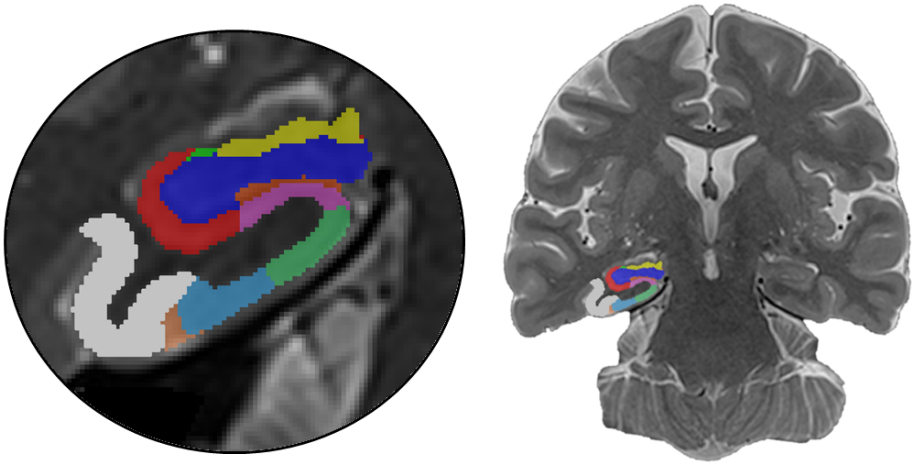

Figure 5. Hippocampal subfields, as segmented by the ASHS protocol (Yushkevich et al., 2010). ● Cornu ammonis 1 (CA1); ● Cornu ammonis 2 (CA2); ● Cornu ammonis 3 (CA3); ● Dentate Gyrus; ● Subiculum; ● Entorhinal cortex; ● Brodmann area 35 (BA35); ● Brodmann area 36 (BA36).

Head/Body/Tail

To obtain HBT segmentation, the authors modified the protocol of Lindberg and colleagues (2017). MATLAB (2010) was used to calculate the two voxels on the ASHS hippocampal mask with the greatest Euclidian distance between them. A line from the most posterior voxel to the most anterior voxel was created and divided into 20 equally spaced segments, each representing 5% of the longitudinal axis of the hippocampus. Each voxel was projected onto the line and assigned to the corresponding segment. Those segments falling into the 9 most anterior segments were grouped to be the hippocampal head (45%), the next 7 segments as the hippocampal body (35%) and the remaining 4 posterior segments as the hippocampal tail (20%). The visual representation of this can be seen in Figure 5. These percentages were based on the findings of Poppenk and colleagues (2013), who concluded that percentage-based demarcation is appropriately aligned with landmark based methods.

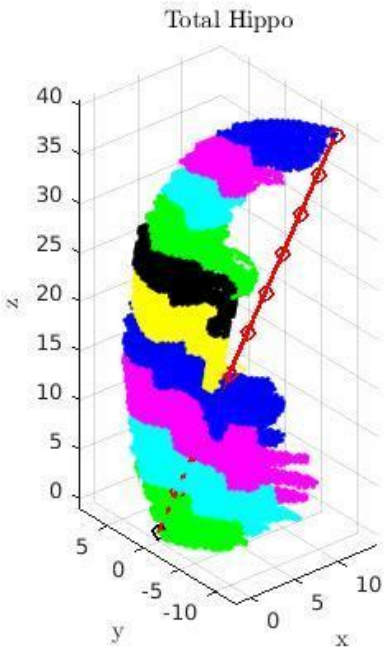

Figure 6. The 20-slice segmentation of the hippocampus across the longitudinal axis used as the basis for head, body and tail segmentation. Image is shown here with 10 slices for visual clarity; however, the actual analysis was split into 20 slices as described in-text.

2.3 Data analysis

Baseline MRI data was normalised to intracranial volume (ICV) to account for head size. Comparisons between baseline and follow-up MRI data were conducted using the raw data as ICV should be stable within individuals, allowing each participant to act as their own control. Visual inspection of the data showed that the ICV of two participants differed beyond what would be expected between baseline and follow-up. These participants were therefore excluded from analyses comparing MRI changes between the two time points. For all other participants there was a strong correlation between baseline and follow-up ICVs (r = .956, p = <.001). Novel measures of tissue integrity were quantified by T2 relaxometry as outlined in Figure 4. In this dissertation, T2 distribution refers to the standard deviation of T2 relaxation time. Statistical data analysis was performed using SPSS v23 (IBM Corp., 2016).

3. Results

3.1 Patient group differences

3.1.1 Volumes

Baseline MRI data was segmented into subfields and head, body and tail. To investigate whether segmenting the hippocampus was better at distinguishing patient groups than whole hippocampal volume, a linear mixed effects model was used with Bonferroni corrected post hoc tests for significant findings.

In each mixed-effects model, subject ID was entered as a random effect in order to account for within-individual factors. The age variable was mean standardised for all analyses (ZAge). ZAge was also added as a random effect. Group, subregion (segmented into either HBT or subfields) and ICV were added as fixed effects. Group (HC/SCD/MCI) and subregion (segmented into either H/B/T or the subfields: CA1, CA2, CA3, dentate gyrus and subiculum) were factors in the model, with ZAge and ICV as covariates. Corresponding volume was the dependent variable.

For HBT subregions, the model revealed a significant main effect of group, $F(2,144.643) = 30.309$, $p < .001$, and a significant main effect of HBT subregion volume, $F(2,129.090) = 858.126$, $p < .001$. Based on this HBT segmentation data, Bonferroni corrected post hoc tests showed that volumes between the HC and SCD groups did not significantly differ ($p = .593$), but that HBT volumes in the MCI group were significantly lower than both the HC and SCD groups (both *p*s <.001). There was no HBT volume x group interaction, $F(4,129.090) = 0.980$, $p = .421$. There was also a significant main effect of ICV, $F(1,114.747) = 26.361$, $p < .001$.

Similarly, for subfields, the model confirmed a significant main effect of group, $F(2,166.740) = 24.440$, $p < .001$, and a significant main effect of subfield volume, $F(4,278.315) = 2070.347$, $p < .001$. Based on this subfields data, Bonferroni corrected post-hoc tests revealed no significant difference in volumes between the HC and SCD groups ($p = .824$), however there was a significant difference in volumes between those two groups and MCI (both *p*s <.001). There was a significant subfield volume x group interaction, $F(8,278.315) = 9.211$, $p < .001$. Again, there was a significant main effect of ICV = $F(1,136.481) = 16.886$, $p < .001$.

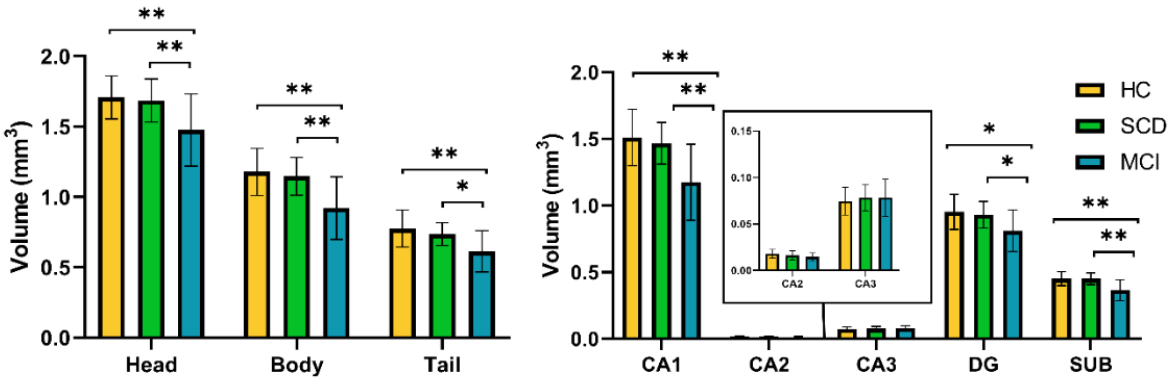

*Figure 7. Graph of volumes by group. HC = Healthy control; SCD = Subjective cognitive decline; MCI = Mild cognitive impairment; DG = Dentate gyrus; SUB = Subiculum. ** = p = ≤.001, * = p = ≤.05.*

This led to investigation into the group differences within each HBT subregion. Individual one-way ANOVAs were conducted to understand the differences in subregion volume between patient groups.

Table 3 shows the differences in head, body and tail volumes between groups. All three subregions along the longitudinal axis were significantly different between the MCI group and those in the HC and SCD groups (all *p*s <.004). Across the subregions HCs and those with SCD were not significantly different.

Table 3. Statistics from a series of one-way ANOVAs investigating patient group differences in the volumes of hippocampal head, body and tail subregions.

	HC	SCD	MCI	ANOVA	
	M ± SD			*F(2,65)*	*p*
Head**	1.71 ± 0.15	1.69 ± 0.15	1.48 ± 0.26	9.576	<.001
Body**	1.18 ± 0.17	1.15 ± 0.13	0.92 ± 0.22	13.451	<.001
Tail**	0.78 ± 0.13	0.74 ± 0.08	0.61 ± 0.15	10.076	<.001

Between groups, significant differences were found via repeated measures ANOVA within CA1, dentate gyrus and subiculum (see Table 4). Tukey's post-hoc test revealed that in CA1, dentate gyrus and subiculum, those with MCI were significantly different from those with SCD and HCs (all *p*s < .01), however across HBT subregions, HCs were not significantly different to those with SCD.

Table 4. Statistics from a series of one-way ANOVAs investigating patient group differences in the volumes of hippocampal subfields.

	HC	SCD	MCI	ANOVA	
		M ± SD		*F(2,65)*	*p*
CA1**	1.51 ± 0.21	1.47 ± 0.16	1.18 ± 0.29	14.360	<.001
CA2	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	3.153	.049
CA3	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.382	.684
Dentate Gyrus*	0.95 ± 0.13	0.93 ± 0.10	0.81 ± 0.16	7.177	.002
Subiculum**	0.45 ± 0.05	0.45 ± 0.05	0.37 ± 0.08	14.600	<.001

A further one-way ANOVA revealed that whole hippocampal volume also differs by group, $F(2,65) = 12.554$, $p < .001$. This showed a similar pattern to subfields, where HC and SCD groups did not significantly differ, but HC/SCD significantly differed from the MCI group (both *p*s <.001)

3.1.2 T2 distribution

In this report, T2 distribution is quantified as the standard deviation of T2 relaxation time. A linear mixed effects model was conducted to investigate the differences between patient groups in T2 distribution within different subregions, as above but using T2 as the dependent variable. Using the HBT segmentation data, the linear mixed effects model revealed a significant main effect of group, $F(2,138.889) = 7.454$, $p = .001$. Bonferroni corrected post hoc tests showed that T2 distribution between the HC and SCD groups did not significantly differ ($p = .384$) and neither did MCI and HC T2 distributions ($p = .063$), but that T2 distribution in the MCI group was significantly wider than the SCD group ($p = .001$). A significant main effect of HBT subregion T2 was also shown, $F(2,138.886) = 96.053$, $p < .001$. There was no significant HBT subregion x group interaction, $F(4,138.886) = 1.907$, $p = .113$. There was also a significant main effect of ICV, $F(1,115.367) = 11.436$, $p = .001$.

For the subfield data, the linear mixed effect model confirmed a significant main effect of group, $F(2,132.797) = 10.188.$, $p < .001$. Bonferroni corrected post hoc tests showed that T2 distribution between the HC and SCD groups did not significantly differ ($p = 1$), but that subfield T2 distribution in the MCI group were significantly wider than both the HC and SCD groups (both *ps* ≤.001). There was also a significant main effect of subfield T2, $F(4,232.340) = 76.475$, $p < .001$. A significant subfield x group interaction was also found, $F(8,232.340) =$

4.714, p <.001. Again, there was a significant main effect of ICV, $F(1,95.495) = 15.139$, p <.001.

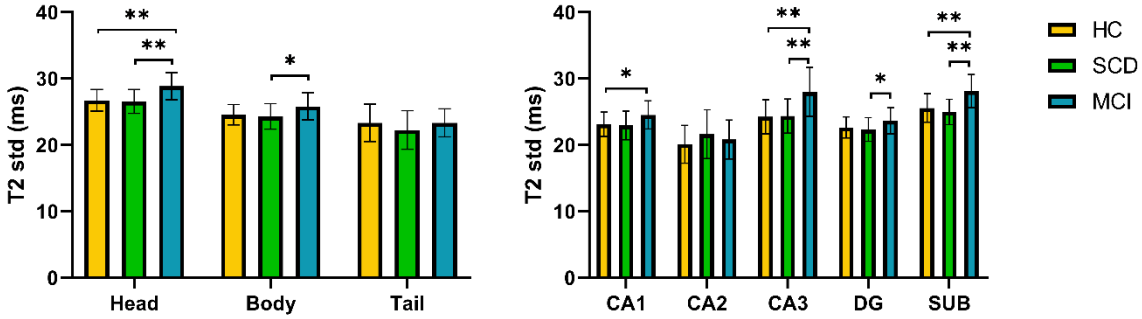

*Figure 8. Graph of T2 distribution by group. HC = Healthy control; SCD = Subjective cognitive decline; MCI = Mild cognitive impairment; DG = Dentate gyrus; SUB = Subiculum. ** = p = ≤.001, * = p = ≤.05.*

These findings prompted a series of one-way ANOVAs, to investigate groupwise comparisons in the T2 distribution of each subregion. Within HBT segments, significant differences in T2 distribution were reported in the head and body of the hippocampus between patient groups, but not in the tail. The head showed the strongest effect, with effect size decreasing along the longitudinal axis (see Table 5). The more posterior the hippocampal subregion, the less T2 distribution differs between the MCI group and HCs/SCDs. Tukey's post-hoc test found that the MCI group differed from HCs and the SCD group in the head (both *p*s < .001). In the body MCIs were significantly different from SCDs (p = .021) but not HCs (p = .063). In both the head and body, HCs and SCDs were not significantly different.

Table 5. Statistics from a one-way ANOVA investigating patient group differences in the T2 distribution of hippocampal head, body and tail subregions.

	HC	SCD	MCI	ANOVA	
		M ± SD		*F(2,65)*	*p*
Head**	26.71 ± 1.63	26.56 ± 1.80	28.84 ± 2.04	10.214	<.001
Body*	24.54 ± 1.53	24.30 ± 1.92	25.83 ± 2.06	4.189	.019
Tail	23.32 ± 2.82	22.26 ± 2.90	23.33 ± 2.11	1.248	.294

Within subfields of the hippocampus, T2 distribution was wider in the MCI group for all subfields except CA2. Tukey's post-hoc test was performed for the significantly different

subfields. Across these subfields the MCI group was significantly different from HCs and the SCD group (all *p*s = <.05), however HC and SCD were not significantly different from each other.

Subiculum, which is thought to be affected in early stages of AD, showed a particularly large effect size (see Table 6). Entorhinal cortex and BA35 were also investigated as they are thought to be similarly involved in early pathology. These regions also showed significant differences between groups with Tukey's post hoc tests showing that both regions did not significantly differ between HCs and SCDs, but that the MCI was significantly different from the HC and SCD groups (both *p*s <.001).

Table 6. Statistics from a one-way ANOVA investigating patient group differences in the T2 distribution of hippocampal subfields.

	HC	SCD	MCI	ANOVA	
	M ± SD			*F(2,65)*	*p*
CA1*	23.11 ± 1.84	22.95 ± 2.18	24.53 ± 2.12	3.716	.030
CA2	20.10 ± 2.85	21.62 ± 3.67	20.82 ± 3.22	1.374	.260
CA3**	24.25 ± 2.56	24.36 ± 2.57	28.00 ± 3.68	10.964	<.001
Dentate Gyrus*	22.64 ± 1.57	22.33 ± 1.78	23.64 ± 1.96	3.154	.049
Subiculum**	25.56 ± 2.16	24.97 ± 1.91	28.12 ± 2.50	12.389	<.001
Entorhinal Cortex**	24.43 ± 2.09	24.49 ± 1.81	28.96 ± 4.45	16.780	<.001
BA35**	22.10 ± 2.05	22.58 ± 2.28	25.80 ± 3.27	13.061	<.001

Due to the lack of meaningful difference between the HC and SCD groups, these participants were pooled for subsequent analyses (a non-MCI group: N=49 and an MCI group: N=19).

3.1.3 Group classification

To test group classification abilities of each subregion, logistic regressions for subregion volume and T2 distribution were conducted with the pooled groups. As shown in Table 7, only hippocampal body volume was able to classify groups along the longitudinal axis, whilst no subfields reached significance. The volume of the whole hippocampus showed the largest effect, suggesting whole hippocampal volume is the optimum measure.

Table 7. Statistics from logistic regressions investigating the classification of groups by subregion volume.

Volume ROI	b	Wald χ^2 (1)	*p*
Head	-0.567	0.041	.840
Body*	-17.913	5.398	.020
Tail	17.006	2.934	.087
CA1	-5.672	2.595	.107
CA2	14.625	0.022	.881
CA3	25.832	1.104	.293
Dentate gyrus	3.364	0.472	.492
Subiculum	-10.674	1.179	.278
Whole hippocampus*	-2.982	13.329	<.001

T2 distribution within the hippocampal head classified the non-MCI and MCI groups well, whilst T2 distribution in the body and tail could not. Only CA3 and subiculum could classify the groups when using subfield segmentation. Whole hippocampal T2 distribution also classified patient groups, with a larger effect size than any subregion. Statistics from the logistic regression can be seen in Table 8.

Table 8. Statistics from logistic regressions investigating the classification of groups by subregion T2 distribution.

T2 ROI	b	Wald χ^2 (1)	*p*
Head*	0.905	7.840	.005
Body	-0.035	0.012	.914
Tail	-0.272	1.858	.173
CA1	0.010	0.001	.978
CA2	-0.030	0.061	.805
CA3*	0.331	4.792	.029
Dentate gyrus	-0.903	2.853	.091
Subiculum*	0.858	6.867	.009
Whole hippocampus*	0.538	9.613	.002

Receiver operating characteristic (ROC) curves were used to show the specificity and sensitivity of subregions volume as a test for presence of MCI. These are shown in Figure 8. Results reported above are mirrored in the ROC analysis in terms of significant differences translating into ability to classify patient groups. Area under the curve (AUC) statistics support specific regions and measures as particularly useful at classifying groups (see Table 9). Subiculum volume and T2 were the best classifiers, with large AUCs (both .814). Other regions that approached an AUC of .8 were body and CA1 volumes and T2 distribution in the head and CA3.

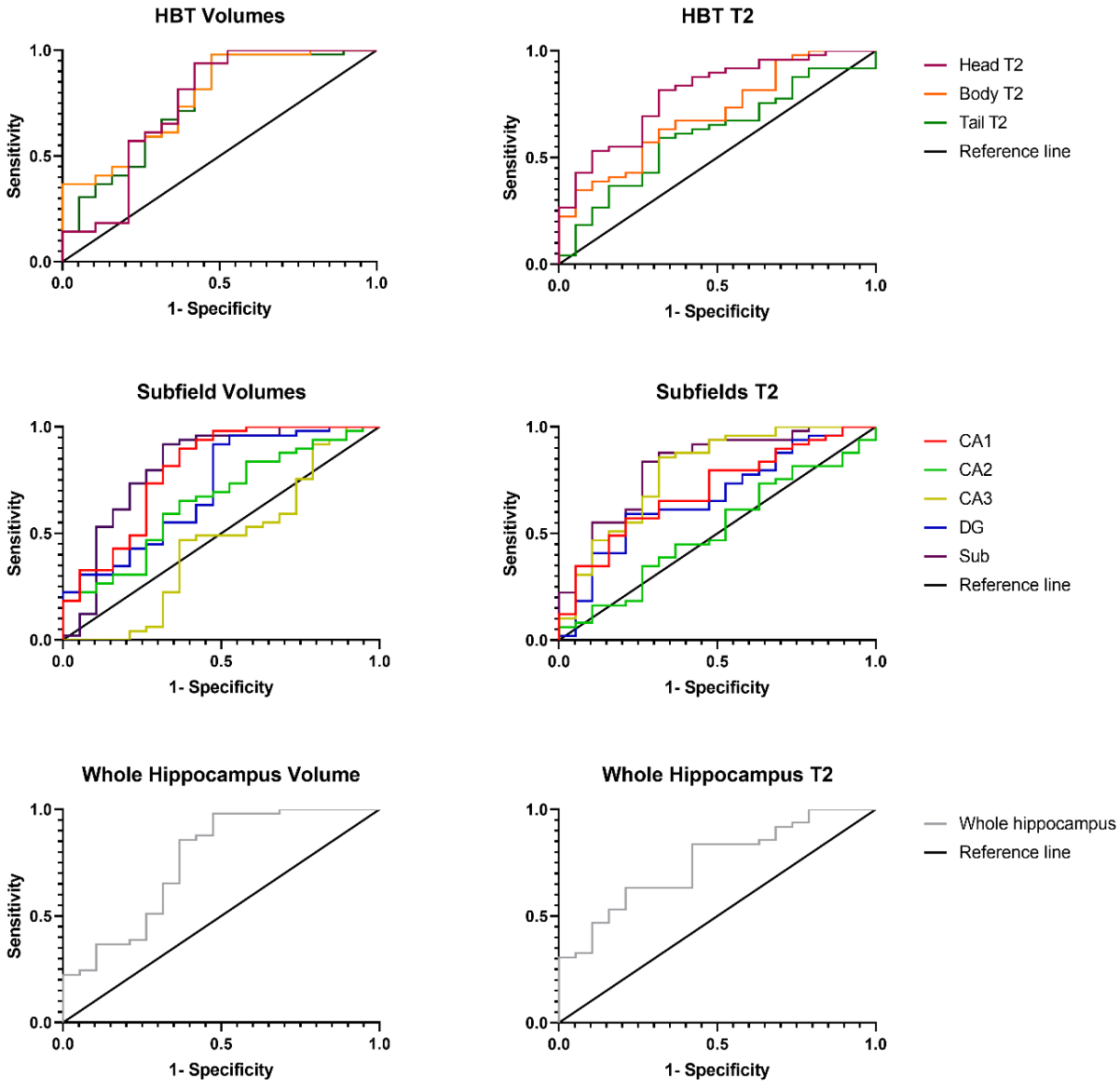

Figure 9. Receiver operating characteristic (ROC) curves showing the specificity and sensitivity thresholds for volumes and T2 distribution when used to classify patient group.

Table 9. Statistics from the ROC curves, showing area under the curve (AUC), standard error (SE) and significance for each subregion measure.

	AUC	SE	*p*
Head volume*	.747	.079	.002
Body volume**	.779	.064	<.001
Tail volume*	.753	.071	.001
CA1 volume**	.795	.068	<.001
CA2 volume	.653	.074	.051
CA3 volume	.450	.088	.525
Dentate gyrus volume*	.711	.074	.007
Subiculum volume**	.814	.069	<.001
Whole hippocampus volume*	.758	.072	.001
Head T2**	.797	.059	<.001
Body T2*	.701	.069	.010
Tail T2	.609	.074	.165
CA1 T2*	.702	.068	.010
CA2 T2	.512	.078	.875
CA3 T2**	.792	.066	<.001
Dentate gyrus T2*	.670	.073	.030
Subiculum T2**	.814	.060	<.001
Whole hippocampus T2*	.749	.063	.002

3.2 Vulnerability to change

Table 10 outlines the changes that were seen in volume and T2 distribution across all participants after a follow-up period of one year. Percentage change was calculated to account for the differential baseline volumes of subregions. T2 distribution was also converted to percentage for consistency. Volume change ranged from -10% in CA2 to +1.9% in CA3, meanwhile T2 distribution change ranged from -4.5% in CA2 to +2.8% in the hippocampal tail. This may be due in some part to measurement error, with small subregions such as CA2 being particularly prone to inflated percentage change. As outlined previously, an increase in T2 distribution here reflects a lower proportion of healthy tissue as this suggests deviation from the norm. A widening of the distribution suggests unhealthy dense tissue (i.e. iron deposits) or unhealthy sparse tissue (i.e. fluid filling atrophy sites).

Table 10. Mean change by subregion.

N = 47	Volume change (M ± SD)	T2 distribution change (M ± SD)
Head	-1.0% ± 5.6%	0.4% ± 5.6%
Body	0.7% ± 6.7%	0.9% ± 5.4%
Tail	0.7% ± 7.4%	2.8% ± 8.1%
CA1	0.6% ± 7.3%	1.4% ± 6.6%
CA2	-10% ± .18.9%	-4.5% ± 17.5%
CA3	1.9% ± 20.0%	2.3% ± 11.5%
Dentate gyrus	-1.2% ± 4.1%	0.7% ± 6.2%
Subiculum	0.4% ± 4.4%	1.0% ± 7.9%

3.2.1 Relationship with baseline cognition

Pearson's correlation coefficients were conducted between each subregion volume and T2 distribution and cognitive performance at baseline (as measured by ACE-III total score) to investigate the impact of brain changes of clinical presentation. Whole hippocampus volume correlated positively with ACE-III score (r = .546, p = <.001), however slightly larger correlations were seen in subregions: hippocampal head and body, CA1 and subiculum (see Table 11). Whole hippocampus T2 distribution at baseline was moderately negatively correlated with cognitive performance at baseline (r = -.432, p = <.001). Again, it is posited

that wider T2 distribution signals unhealthy tissue, that is either pathologically dense or sparse. Again, some regions showed slightly stronger negative correlations: hippocampal head, CA3 and subiculum (see Table 11).

Table 11. Correlations of subregion measures at baseline with cognitive score (Addenbrooke's Cognitive Examination-III) at baseline.

N = 68	*r*	*p*
Head volume**	.502	<.001
Body volume**	.542	<.001
Tail volume**	.489	<.001
CA1 volume**	.555	<.001
CA2 volume*	.310	.010
CA3 volume	-.041	.742
Dentate gyrus volume**	.468	<.001
Subiculum volume**	.506	<.001
Head T2**	-.489	<.001
Body T2*	-.366	.002
Tail T2	-.122	.323
CA1 T2*	-.364	.002
CA2 T2	.009	.942
CA3 T2**	-.565	<.001
Dentate gyrus T2*	-.324	.007
Subiculum T2**	-.493	<.001

3.2.2 Relationship with cognitive decline

Following the finding that selected subregion volume and T2 correlated with cognitive score at a single time point, analyses were conducted to investigate the hypothesis that these biomarkers would predict cognitive decline over a period of one year. No such relationship was found; Pearson's correlation coefficients showed no correlations with baseline volume or T2 in any subregion or with whole hippocampus volume or T2. Whole hippocampus

volume was not significantly correlated with cognitive decline over the year (r = -.052, p = .708). Statistics from the correlational analyses between T2 distribution and cognitive decline can be seen in Table 12. T2 distribution did not significantly correlate with cognitive decline at the whole hippocampus level (r = .125, p = .366), nor within any subregions.

Table 12. Correlations of subregion measures at baseline with cognitive decline over a period of 1 year (measured by Addenbrooke's Cognitive Examination-III).

N = 47	*r*	*p*
Head volume	.013	.924
Body volume	-.114	.413
Tail volume	-.090	.516
CA1 volume	-.123	.376
CA2 volume	-.149	.283
CA3 volume	.211	.126
Dentate gyrus volume	.005	.970
Subiculum volume	-.232	.092
Head T2	.156	.261
Body T2	.135	.330
Tail T2	-.013	.926
CA1 T2	.050	.720
CA2 T2	-.121	.382
CA3 T2	.195	.158
Dentate gyrus T2	.032	.819
Subiculum T2	.122	.379

Further correlations were carried out to check these relationships between the pooled Non-MCI and MCI groups. No significant correlations were found between subregion volumes nor T2 and cognitive decline over the year, in either of the pooled groups. The exception to this was CA2 volume in those with MCI. In those without MCI, whole hippocampus volume was not correlated with cognitive decline (r = .046, p =.780), nor was whole hippocampus T2 (r =

.202, p = .219). In those with MCI, whole hippocampus volume (r = .030, p =.944) nor T2 were correlated with cognitive decline (r = -.284, p = .495).

Table 13. Correlations of subregion measures at baseline with cognitive decline over a period of 1 year (measured by Addenbrooke's Cognitive Examination-III), split by group: Non-MCI/MCI as outlined in previous analyses.

	Non-MCI (*N*=39)		MCI (*N*=8)	
	r	*p*	*r*	*p*
Head volume	-.013	.937	-.271	.516
Body volume	.012	.944	.185	.661
Tail volume	-.009	.956	.268	.520
CA1 volume	-.053	.750	.206	.624
CA2 volume	.036	.826	.748*	.033
CA3 volume	.005	.977	-.492	.216
Dentate gyrus volume	-.032	.848	-.112	.792
Subiculum volume	-.035	.833	.391	.338
Head T2	.122	.461	-.239	.569
Body T2	.200	.223	-.339	.412
Tail T2	.207	.206	-.013	.976
CA1 T2	.273	.093	-.365	.374
CA2 T2	.250	.124	.092	.829
CA3 T2	-.002	.989	.012	.978
Dentate gyrus T2	.213	.194	.037	.931
Subiculum T2	.126	.444	-.087	.838

4. Discussion

This research aimed to investigate brain changes in people with prodromal AD, to attempt to validate the best method of segmenting hippocampus for use in SCD/MCI/AD and reveal patterns of pathology. There were measurable differences found between participants who reported SCD and HCs. Those with MCI had smaller whole hippocampal volumes than those without MCI. These differences were also found in the subregions CA1, DG and subiculum and also in all three longitudinal segments of the hippocampus. Somewhat similarly, T2 distribution was wider in those with MCI in whole hippocampus, CA3 and subiculum, DG and CA1. Follow up analysis showed differences in the entorhinal cortex and BA35, but this was beyond the scope of this report. Group classification analyses showed that whole hippocampal volume and T2 were sufficient to classify MCI and non-MCI groups. ROC analysis highlighted subiculum volume and T2 as equally the best classifiers of group membership. Data collected after a period of one year showed a large decrease in CA2 volume. T2 distribution became wider over the year in the hippocampal tail. Whilst general cognitive ability was correlated to some volumes (whole hippocampus, head, body, tail, subiculum) and T2 distributions (head, CA3, subiculum) at that same time point, no volume nor T2 could predict decline in cognitive ability a year later.

4.1 SCD is subjective and heterogeneous

The study found no significant differences in volume nor T2 distribution between those with SCD and HCs. This is to some extent expected as the definition of SCD is that there is no objective sign of disease, in contrast to the individual's self-report of memory deficits. It was hoped that there would be structural evidence of a prodromal disease stage of MCI. This lack of difference between patient groups constrains that any conclusions drawn about early AD in this report refer to the MCI-stage of disease and not earlier pathology. This is concordant with some previous literature that has found no hippocampal volume differences in those with SCD (Jorm et al., 2004; Saykin et al., 2006). Saykin and colleagues report hippocampal volume loss correlated with extent of memory problems. It is likely that if any damage occurs to the hippocampi of those with SCD, it is subtle and corresponds with an equally subtle impairment that is not detected by clinically used tests. It is unlikely that hippocampal volumetry, or as shown in this study T2 relaxometry as a proxy for tissue health, is sensitive enough to detect changes in very early AD, before the objective manifestation of symptoms.

A wider issue in the study of SCD is defining a potential disease stage that is by nature subjective. Self-report measures of memory ability have been established as a poor

representation of objective performance (Crumley, Stetler & Horhota, 2014). There are also many different causes and mediators of SCD, suggesting it is not a homogenous group. SCD is also a complex condition, affected by presence of the apolipoprotein E genotype (APOE), psychiatric symptoms and poor physical health (Jorm et al., 2004). These factors may reflect not only biological risk factors, but also the self-reporting of these individuals, for example with a family history of AD (higher chance of APOE), who exhibits anxiety behaviours may be more likely to report concerns about their memory. This heterogeneity of the patient group may explain the mixed structural findings that have been seen in this area of research. This is also a characteristic of MCI. Research describes that two years after presenting with MCI, 11.1 to 21.2% of individuals remain stable and 33.3 to 55.6% no longer have MCI, with half of those individuals returning to their previous levels of cognitive ability (Ganguli, Dodge, Shen & DeKosky, 2004).

4.2 Whole hippocampus is a good standard for detecting disease, but head tissue health may be preferentially affected in those with prodromal AD

Investigating MCI and non-MCI groups in the current study revealed differences across hippocampal head, body and tail volumes. This is also the case with all subfields, except for CA2 and CA3. This finding is likely to be due to the small size of these subfields compared to others. Proportional changes to these regions are likely harder to detect at such a fine scale. Whole hippocampal volume also shows significant groupwise differences. This suggests that when investigating volumes there is no advantage to using either of the segmentation methods, above what is already a clinical standard for identifying likely AD – whole hippocampal volume.

However, when investigating which brain regions are useful for classifying those with MCI and those without, it is the subiculum that is particularly powerful. Both volume and T2 were strongly sensitive and specific tests of group membership. The subiculum, along with CA1, is thought to be particularly affected in AD, with an average of 24% neuronal loss and 48% respectively (West, Coleman, Flood & Troncoso, 1994; West, Kawas, Stewart, Rudow & Troncoso, 2004). This suggests a potentially more diagnostically accurate test for MCI than the current whole hippocampal measure. This is also a reasonable adjustment to clinical practice, as a T2-weighted scanning sequence could be added to a standard scan. Programmes for segmentation such as ASHS are also semi-automatic, so there is little expertise or labour needed to use it.

Whether T2 relaxometry could be used to add another measure of tissue state was a key question in this research. Previous evidence has shown the significance of early T2 changes

in the hippocampal head, CA1 and subiculum (e.g. Gordon et al., 2013). A similar pattern was found in T2 distribution with whole hippocampal T2 being a useful measure of difference between MCI and non-MCI patient groups. Distinctively, there was a pattern of strong T2 distribution differences in the hippocampal head, with the effect decreasing along the longitudinal axis of the hippocampus. This suggests an early pathology localised to the hippocampal head. However, over a period of one year, the biggest percentage change in T2 occurred in the hippocampal tail. This differential pattern along the longitudinal axis could suggest very early pathology occurs in the hippocampal head, but a later rapid change in the tail. Some work suggests the opposite of this finding: that posterior hippocampal areas are more affected than anterior, specific to AD (Likeman et al., 2005). This is a finding that should be explored further. It is possible that the hippocampal tail is not yet significantly affected in the early stages of disease and that MCI occurs at the same time as an accelerated pattern of normal ageing in the anterior hippocampus. The finding here that tail T2 distribution changes most over the year may support this later posterior pathology.

This research suggests that T2 relaxometry can be a useful tool that provides more information about the health of tissue within the hippocampus in addition to volume. Atrophy is a consequence of pathology, and here it was found that across the longitudinal axis there was no differential pattern of volume loss. However, when investigating tissue health through T2 distribution, a potential pattern of early AD pathology emerges, most marked in the hippocampal head and gradually decreasing towards the posterior hippocampus. Changes seem to occur largely in CA1 and DG, but for segmentation purposes this is demonstrated best by investigating the hippocampal head, using HBT segmentation.

4.3 MRI measures correlate with cognitive ability but cannot predict decline

This research explored general cognitive ability in relation to these brain changes. Hippocampal measures at baseline were correlated to cognitive score at that time point, except CA3 volume and CA2 and hippocampal tail T2 distributions. This, unsurprisingly, shows that the hippocampus correlates with memory performance on standardly used tests. The lack of relationship between the hippocampal tail and cognitive score gives further evidence for the importance of anterior regions of the hippocampus in memory performance in prodromal AD. However, no measures were able to predict decline in the current study. Neither volumes nor T2 distribution were linked to cognitive decline over 1 year. This was also the case when investigating the relationship within the Non-MCI and MCI groups. CA2 volume was the only measurement at baseline that correlated with cognitive decline over the 1-year period. At present, it is unknown why this would be the case. This may be due to the

small size of the subfield being more prone to measurement error and prompting a type 1 error.

It is likely that a longer follow up period would yield more information about whether it is possible to predict decline using hippocampal volume and T2 measures. For the subset of MCI patients who convert to AD, the average time taken for this conversion is 15 months (Hye et al., 2014). As such it is likely that the participants here, showing none or early signs were even further away from a potential AD diagnosis. Future research should employ a follow-up period of longer than a single year, as there is evidence to suggest that significant changes would take longer to occur than in the timeframe of the current study. If an early biomarker is attainable, it is important to ascertain the stage at which this would be most detectable and fruitful.

4.4 Potential confounds

The different segmentation methods outlined above highlight the varied methods for segmentation between timepoints, across labs and within segmentation protocols. Wisse and colleagues (2017), operating as the Hippocampal Subfields Group, have called for harmonisation of these methods. Currently different researchers use different atlases, which employ different terminology, e.g. DG can be seen as CA4 (Duvernoy, 2005), the hilus (West & Gundersen, 1990) or part of CA3 (Insausti & Amaral, 2012). There is some significant disharmony in segmentation protocols and regional boundaries (see Yushkevich et al., 2015), particularly in head and CA1 and subiculum. This is problematic when using hippocampal volumetry for diagnosis of disease or as a proxy marker for disease progression and when comparing studies to draw wider conclusions about the hippocampus. There is a need for harmonised protocols across laboratories to improve the diagnostic value and clinical utility of hippocampal imaging.

ASHS was chosen to segment the subfields of the hippocampus as it uses T2 scans with a higher resolution to demarcate the subfields and is stable across raters (Pluta et al., 2012). However, 11 participants were excluded from the study as the ASHS package failed to mask the hippocampus correctly. Despite using MCI training masks, these failures tended to be on scans showing the most atrophy. This created a bias in the study in that the results do not include the most damaged participants. In addition, there was also a lower retention rate of participants with MCI over the year follow-up period. Many of the participants who did not return because they were too impaired or ill. These factors are likely to have skewed the resulting data and conclusions to represent those who have memory problems that are less severe.

In addition, the resulting participant group was used to draw conclusions about early AD, but their memory problems were not necessarily attributable to prodromal AD. The participants included had some level of amnestic impairment, but this could have been caused by a number of factors. Variables such as body mass index, hypertension, IQ and medication were not taken into account in this study. There is strong evidence that hypertension in older adults, for example, results in increased hippocampal and whole brain atrophy (Wiseman et al., 2004). Future studies should control for these factors. Disorders such as depression or anxiety can also result in cognitive changes (Bierman, Comijs, Jonker & Beekman, 2005) and should be included in any further analyses.

It is also important to consider that brain damage does not always have a direct relationship with performance and how cognitive reserve and compensation can affect this. This theory suggests that some individuals are able to maximise their performance and recruit other brain networks better than others, resulting in a delayed presentation of symptoms (Stern, 2002). Intellectual activities implicated in building up cognitive reserve are even a protective factor, lowering conversion of SCD to MCI/AD (Bessi et al., 2018).

It is also possible that participants in the current study were exhibiting early stage symptoms of other types of dementia or had a transient memory problem. This may be a contributing reason as to why some studies, including the current work find that the hippocampal head is most affected in both in healthy ageing and in AD (e.g. Gordon et al., 2013) and some studies report the same region as being spared (e.g. Lindberg et al., 2017). Research into the subregions of the hippocampus in AD need to consider the methodological differences that underpin conflicting results.

4.5 Future research

MRI currently provides a reasonable tool for use in clinical diagnosis of AD. MRI is already available in medical settings, is relatively cheap and has a short acquisition time, so is a promising method for investigating prodromal AD. Hippocampal imaging and segmentation should be employed in a longitudinal study, tracking biomarkers and changes across a much longer follow-up period than was employed here. As shown in the current study, T2 relaxometry may also provide more information about the early changes in hippocampal tissue. Together with a larger sample size, a longitudinal study would gain power to identify smaller changes in prodromal AD. Here, the importance of T2 in the entorhinal cortex and BA35 in MCI was briefly examined and should be investigated further. Future research should include these regions, in addition to the hippocampus proper.

It may be also fruitful to investigate the hippocampus through the frame of connectivity. The hippocampus is a brain region in the medial temporal subsystem of the default mode network (DMN; Cordes et al., 2001). The DMN is a task negative network, that is one in which the components are simultaneously active when the brain is supposedly ‘at rest’ and deactivated when completing cognitive tasks. This deactivation occurs in response to working memory tasks and its suppression during memory encoding is instrumental in successful long-term consolidation (Lefebvre & D’Angiulli, 2019). Within the medial subsystem of the DMN it has been suggested that there are two distinct pathways. The first shows the body of the hippocampus and posterior parahippocampal cortex, correlated with the lateral parietal cortex, posterior cingulate, retrosplenial cortex and ventral medial prefrontal cortex. The other shows an activation of the hippocampal head and the perirhinal/entorhinal cortices with areas of the lateral temporal cortex extending into the temporal pole (Kahn, Andrews-Hanna, Vincent, Snyder & Buckner, 2008).

This is a possible frame through which to view the hippocampus. Functional segmentation has proved useful in other brain regions such as the thalamus and cerebellum (see Zhang et al., 2008); O'Reilly, Beckmann, Tomassini, Ramnani & Johansen-Berg, 2009). In addition to the gray matter volume of subregions of the hippocampus, functional connectivity of these regions may be enlightening in the search for early AD pathology. There is evidence that dysfunctional connectivity of the DMN is present in early AD (see Stein et al., 2000; Wang et al., 2006; Sorg et al., 2007). Functional connectivity of the hippocampus along its longitudinal axis can be a potential biomarker of early AD. Segmentation may not be more useful than whole hippocampal measures for diagnosing MCI/AD but when segmentation is used alongside T2 distribution as a proxy for tissue health, a pattern emerges. This in combination with functional data is a likely source of vital information about the early stages of AD and its progression.

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Appendices

Appendix A: Baseline protocol

Session 1 **Participant ID: _____**

Time started (/finished)	Task (time estimate)
	Consent Forms (10m)
	Informant CDR interview (20m) T-ADLQ questionnaire
	RBMT-3 (30m) **Version:**
	Story Learning & Immediate Recall (10m)
	CVLT Learning & Immediate Recall (10m) **Version:**
	Rey Complex Figure Task – Copy & Immediate Recall (10m) **Version:**
	Story Recall (5m)
	CVLT Recall & Recognition (5m)
	Participant CDR interview (10m)
	RCFT Recall & Recognition (5m)
	Confirmation of next appointment

Total Testing Time:

Session 2 (4 weeks)

Time started (/finished)	Task (time estimate)
	MRI Screening Forms (10m) (Initial & Second)
	MRI Scan (40m)
	Story Recall (5m)
	CVLT Recall & Recognition (5m)
	RCFT Recall & Recognition (5m)
	ACE-III (15m) **Version:**
	PAL (20m) **Version:**
	Questionnaires – IADL, BCRS, DASS21
	Payment (5m)

Appendix B: Follow up protocol

Session 1 **Participant ID:** _____

Time started (/finished)	Task (time estimate)
	Consent Forms (10m)
	Informant CDR interview (30m) T-ADLQ questionnaire
	RBMT-3 (30m) **Version:**
	Word List Learning & Immediate Recall (10m)
	PAL (20m) **Version:**
	Participant CDR interview (10m)
	Word List 30m Recall & Recognition (5m)
	Confirmation of next appointment & phone calls

Total Testing Time:

Session 2 (4 weeks)

Time started (/finished)	Task (time estimate)
	MRI Screening Forms (10m) (Initial & Second)
	MRI Scan (40m)
	Prospective Memory – time based 4 min: Phone call 20 min: write down initials & DOB on post-it
	[Word List Recall & Recognition (5m), if after 24h or 7d]
	ACE-III (15m) **Version:**
	Questionnaires – IADL, BCRS, DASS21
	Payment (5m)

*Include some prompt at the end after ~30mins if prospective tasks not remembered

Prospective memory scoring:	
Phone call: time remembered:	
Prospective points:	*Retrospective points:*
Remembered something within 1 minute (2 points)	Remembered correct task (2 points)
Remembered something within 5 minutes (1 point)	Remembered other task (1 point)
Remembered something at some point (0.5 points)	Reminds you of an incorrect task (0.5 points)
Remembered only after prompt (0 points)	Did not remember task (0 points)
Write name & DOB: time remembered:	
Prospective points:	*Retrospective points:*
Remembered something within 1 minute (2 points)	Remembered correct task (2 points)
Remembered something within 5 minutes (1 point)	Remembered other task (1 point)
Remembered something at some point (0.5 points)	Writes something other than name/DOB (0.5 points)
Remembered only after prompt (0 points)	Did not remember task (0 points)

Appendix C: T2 distribution by subregion

Differences in T2 distribution in each subregion across groups. Within HBT, significant difference in T2 distribution between subregions, $F(2,65) = 63.224$, $p = <.001$. Within subfields, $F(2,65) = 41.068$, $p = <.001$.

Table C.

	M	SD
Head	27.25	2.05
Body	24.81	1.92
Tail	22.94	2.69
CA1	23.45	2.13
CA2	20.86	3.22
CA3	25.34	3.32
Dentate Gyrus	22.80	1.82
Subiculum	26.06	2.51

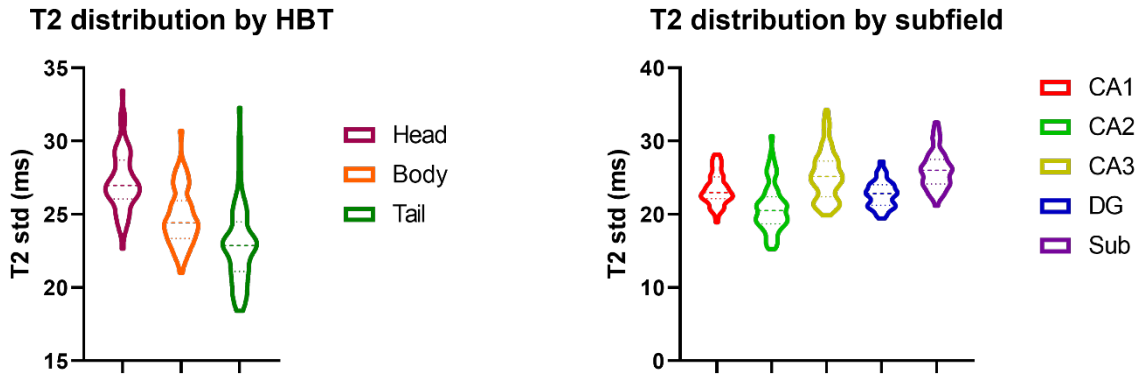

Figure C.

Appendix D: Hemispheric asymmetry

There is also a functional distinction to be made between the left and right hippocampi. Functional imaging studies show selective activation in the left PH when performing verbal tasks (Greicius, Krasnow, Reiss & Menon, 2003). Further fMRI and PET studies, and a developmental case study suggest that the left hippocampus is a region heavily involved in autobiographical memory (Burgess, Maguire & O'Keefe, 2002; Maguire & Mummery, 1999; Maguire, Vargha-Khadem & Mishkin, 2001). This conclusion is supported theoretically as the importance of the left hippocampus in verbal memory is likely to constrain ability to form context-laden narratives necessary to demonstrate a sense of personal experience and history.

A dissociation is seen with spatial memory, which shows activation in the right PH (Maguire, Frackowiak & Frith, 1997). This verbal-spatial distinction is further supported by Maguire and colleagues' seminal taxi driver study (2000); highly skilled spatial practitioners showed neuroplasticity of the right hippocampus specifically, in response to this environmental demand. Further studies in humans have also suggested a link between the right hippocampus and spatial memory from acquired lesions (Štěpánková, Fenton, Pastalkova, Kalina & Bohbot, 2004) and gene expression specifically in the right hippocampus after spatial learning (Klur et al., 2009).

Cognitive map theory suggests that the two hippocampi work together, each distinctly specialising in spatial and narrative relationships, with temporal information being produced by connections with the frontal lobe to one or both hippocampi (O'Keefe & Nadel, 1978). This connectivity results in a well-rounded episodic memory system in healthy individuals.

Within AD populations, most volumetric research shows pathology in the left hippocampus to a larger extent than the right (Pennanen et al., 2004; Zhao et al., 2019). Left hippocampal volume has also been found to predict conversion to AD from MCI (Zhou, Nakatani, Teramukai, Nagai & Fukushima, 2012). Segmentation in those with MCI has revealed that left CA1, DG, hippocampal head and tail are affected most. Pluta and colleagues (2012) show that left CA1 emerged as a better predictor of patient group than whole hippocampal volume. There is also evidence to suggest that the left subiculum is most correlated to tests of memory ability (Zhao et al., 2019).

However, this is not a conclusive picture. A meta-anaylsis of hippocampal asymmetry in MCI and AD concluded that pathology is bilateral (Shi et al., 2009). In those with MCI and AD, atrophy in both the left and right hippocampus has been found, but there is a tendency for

more damage in the left, especially in those with MCI. In addition, there have also been T2 findings that suggest increased mean T2 specifically in the right hippocampal head and tail (Laakso et al., 1996). This may be due to the issues with using mean T2 as a measure, as presented above. There is still much to learn about T2 relaxation time and what information it can provide in understanding AD.

A paired samples t-test was conducted to investigate whether there were differences in T2 distribution within left and right hippocampi. Right hippocampus T2 distribution (M = 25.67 ± 1.90) was slightly higher than in the left (M = 25.89 ± 2.07) but not significantly, $t(68) = 1.683$, $p = .097$. T2 distribution in the left hippocampal head was significantly larger than the right, suggesting that the left head contained less healthy tissue across the groups. This was not seen in the body nor tail subregions. CA1, dentate gyrus and subiculum also showed a significantly wider T2 distribution in the left hippocampus when compared to the right. See Table D for means, standard deviations and t-tests relating to left and right hemispheres and Figure D for visual representation of this data.

Table 13. Statistics from paired samples t-tests investigating differences between left and right subregions across groups.

	Left hemisphere	Right hemisphere	Paired samples t-test	
	M ± SD		t (68)	p
Head**	27.58 ± 2.27	27.01 ± 2.08	3.435	<.001
Body	24.85 ± 2.18	24.84 ± 1.88	0.061	.951
Tail	22.97 ± 3.18	23.09 ± 2.99	-0.357	.722
CA1*	23.63 ± 2.34	23.27 ± 2.15	2.053	.044
CA2	21.00 ± 4.71	20.60 ± 3.84	.587	.559
CA3	25.37 ± 3.82	25.18 ± 4.06	.366	.716
Dentate Gyrus**	23.27 ± 2.15	22.30 ± 1.84	5.186	<.001
Subiculum*	26.36 ± 2.61	25.82 ± 2.69	2.425	.018

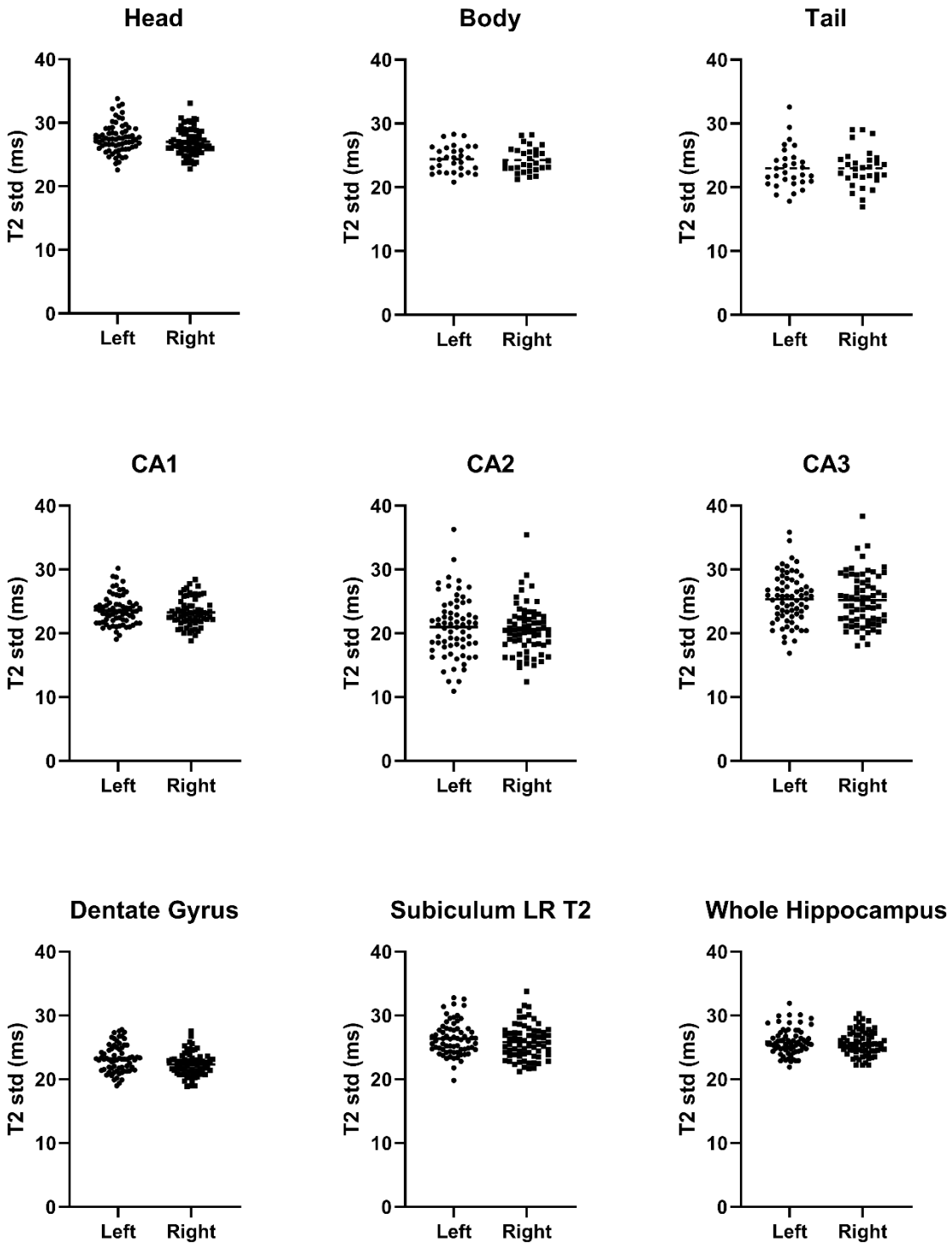

Figure D. Graphical representation of the T2 distribution in the left and right hippocampi, broken down by subregion. The reference line depicts mean.

A mixed ANOVA was conducted to investigate hemispheric T2 distribution by pooled group. Mauchly's test, $\chi^2(0) = 0.00$ indicated that the violation of sphericity was exactly met. There was a main effect of hemisphere, $F(1,66) = 6.299$, $p = .015$. However, there was not a significant hemisphere x pooled group interaction, $F(1,66) = 0.875$, $p = .353$.